

STEROLS OF *TETRASELMIS* (PRASINOPHYCEAE)

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(Received 19 October 1992; accepted 8 January 1993)

Abstract—1. Sterols were identified from 11 isolates of *Tetraselmis*, a unicellular Prasinophyte alga used frequently as food in mariculture.

2. The principal sterol in eight isolates was either 24-methylenecholesterol or 24-methylcholesterol; the latter was determined to be campesterol in all cases.

3. Campesterol is the first 24 α sterol to be reported in the Prasinophyceae.

4. In the remaining three isolates, cholesterol was the principal sterol with smaller amounts of 24-methylenecholesterol and campesterol present; in two of these strains total sterol approached 3% of dry weight.

5. This is the first report of cholesterol as the principal sterol of a Prasinophyte; the C28 sterols found in *Tetraselmis* are the dominant sterols in most Prasinophyceae studied to date.

INTRODUCTION

Tetraselmis is a prasinophyte algal genus which is used widely as a food source in mariculture (Guillard, 1975; Webb and Chu, 1981; Okauchi, 1988; Laing, 1991). In our experiments, *Tetraselmis chui* (clone LB-232) was shown to produce good growth of post-set oysters, and *T. maculata* (clone TTM) produced oyster growth superior to all other phytoplankton species tested in several experiments (Ukeles and Wikfors, 1988; Ukeles *et al.*, 1984; Wikfors *et al.*, 1984, 1990). The oyster is unable to synthesize sterols from smaller molecules (Salaque *et al.*, 1966; Trider and Castell, 1980; Holden and Patterson, 1991), therefore sterols are required in the oyster's diet (Teshima, 1981). Recent studies show a positive correlation between sterol composition of a phytoplankton species and its effectiveness in producing rapid oyster growth (Wikfors *et al.*, 1990).

In studies previous to ours, three species of *Tetraselmis* were examined for sterol content. The major sterol in each species was either 24-methylenecholesterol or 24-methylcholesterol (Ballantine *et al.*, 1979; Lin *et al.*, 1982; Volkman, 1986). Both of the above sterols are found in oysters collected from the U.S. Atlantic coast (Teshima and Patterson, 1980) where sterol composition is reflective of the diet (Berenberg and Patterson, 1981). The C-24 orientation of 24-methylcholesterol has not been determined in the oyster, but in the scallop it has been determined to be a mixture of 24 α and 24 β (Khalil *et al.*, 1980). At one time, algae were thought to contain only 24 β sterols (Patterson, 1971), although recently the diatom

Nitzschia brevivostriis was shown to contain 24 α -methyl-cholesterol (campesterol) (Gladu *et al.*, 1991). It would appear that other phytoplankton must contain 24 α -methylcholesterol to account for the 24 α -methylcholesterol found in the scallop. The orientation at C-24 of 24-methylcholesterol has not been determined for any Prasinophyte (Patterson, 1992).

As recent research has shown that sterol composition of the diet affects oyster growth, and *Tetraselmis* is known to support rapid growth of oysters, knowledge of the sterol composition of *Tetraselmis* strains would be useful in choosing the most effective strains for feeding post-set oysters in intensive aquaculture. This paper reports on the identity and quantity of sterols present in 11 isolates of *Tetraselmis*.

MATERIALS AND METHODS

Algal culture

All *Tetraselmis* cultures were grown axenically (with the exception of clones Rey 2N and TT-Caic, which are bacterized) in enriched natural sea water "E" formulation (Ukeles, 1973) in Fernbach flasks and harvested in the stationary phase as described previously (Gladu *et al.*, 1991). All isolates tested were from the Milford Microalgal Culture Collection where they have been maintained in E medium for 5–40 years. Historic sources of strains, when known, are listed in Table 1.

Harvested cultures were centrifuged, frozen in cold

Table 1. Milford strain designations and source information available for algal cultures analyzed

Milford Strain Designation	Source information
LB-232	<i>Tetraselmis chui</i> , from UTEX (LB-232), isol. Chu, Scotland.
TTM	<i>T. maculata</i> , from Parsons, isol. Parsons, Departure Bay, WA.
Platy-1	<i>T. levis</i> , from Guillard, isol. Guillard, Great Pond, Falmouth, MA.
NOR-5-4-66G	<i>Tetraselmis sp.</i> , isol. Ukeles, Chesapeake Bay, Virginia.
Rey 3	<i>Tetraselmis sp.</i> , via Hayhome, 1987, isol. Reyes, Venezuela, 1975.
Rey 2N*	<i>Tetraselmis sp.</i> , via Hayhome, 1987, isol. Reyes, Venezuela, 1975.
TT-4 Ply 305	<i>T. suecica</i> , via Lewin, 1986, isol. Parke, Plymouth, U.K.
TT-9 RAL	<i>Tetraselmis sp.</i> , from Lewin, 1986, isol. Lewin, Herring Cove, Nova Scotia, Canada.
TT-Caic*	<i>Tetraselmis sp.</i> , isol. Wikfors, 1987, Providenciales, Turks and Caicos Islands, BWI.
TT-3 Ply 429	<i>T. suecica</i> , via Lewin, 1986, from Plymouth Coll.
TT-TAH	<i>Tetraselmis sp.</i> , via Haines, isol. Tahiti (actually a <i>Chlamydomonas</i> species; Ralph Lewin, personal commun.)

*Strain is not axenic.

methanol (-25°C), lyophilized as described previously (Gladu *et al.*, 1991) and stored in a freezer until extracted. Cells from the centrifuged concentrate were diluted volumetrically and counted in an Improved Neubauer Hemocytometer with a light microscope. Dry weights were determined by weighing on an analytical balance a known number of cells collected on a glass fiber filter (Whatman GF/F*), washed with ammonium formate isotonic to the growth medium, and dried in an oven at 80°C . Means of two dry weight determinations were calculated for each species; ranges never exceeded 5% of the mean.

Lipid analysis

Lyophilized samples (0.5–1.0 g) were extracted overnight in a Soxhlet apparatus with CHCl_3 /

*Mention of trade names does not imply endorsement.

methanol (2:1 v/v). The crude lipid extract was then partitioned into ester, free alcohol, and glycoside (polar) fractions by BioSil A column chromatography (Gladu *et al.*, 1991). Ester and glycoside fractions were base- and acid-hydrolyzed, respectively, and liberated sterols were analyzed by capillary gas chromatography on a Varian 3500 gas chromatograph equipped with a $30\text{ m} \times 0.25\text{ mm}$ SPD-1 fused silica column. Sterols were identified and quantified by gas chromatography using a cholesterol standard. Mass spectra were obtained with a Finnigan-MAT Model 4512 gas chromatograph-mass spectrometer equipped with a $30\text{ m} \times 0.32\text{ mm}$ i.d. fused silica capillary column coated with a $0.25\text{ }\mu\text{m}$ film of DB-1. Spectra were collected at 70 eV and at a source temperature of 150°C . Data were recorded and analyzed with an Incos Data System.

RESULTS AND DISCUSSION

Table 2 compares the sterol composition of three species of *Tetraselmis* studied previously with the 11 isolates of this study. Eight of the 11 isolates examined containing the 28-carbon sterols 24-methylencholesterol and/or 24-methylcholesterol as the principal sterols. 24-Methylencholesterol was identified by its GC relative retention time (1.23) and its mass spectrum, which was characterized in particular by a molecular ion at m/z 398 and the presence of a base peak at m/z 314 characteristic of a 24(28) double bond (Rahier and Benveniste, 1989). 24-Methylcholesterol was identified by its relative retention time (1.26) on capillary GC (Gladu *et al.*, 1991) and by its mass spectrum, which was characterized by a molecular ion at m/z 400 and peaks at m/z 385, 382, 367, 315, 289, 273, 261, and 255. 24-Methylcholesterol was predominant in five isolates, and in one isolate 24-methylencholesterol was the principal sterol. In two isolates, 24-methylencholesterol and

Table 2. Sterols of *Tetraselmis*

Species/isolate	mg/g dry wt	fg/cell	Sterol composition*		
			CHOL	24MEC	24MC
<i>Tetraselmis suecica</i> †	1	NA	1	51	48
<i>T. tetrahele</i> ‡	NA	NA	5	58	34
<i>T. chui</i> §	NA	NA	—	—	99
<i>T. chui</i> (LB-232)	1.9	140	—	3	97
<i>T. maculata</i> (TTM)	1.3	140	1	27	72
<i>T. levis</i> (Platy-1)	3.9	204	1	20	75
<i>Tetraselmis sp.</i> (NOR-5-4-66G)	1.2	86	7	14	79
<i>Tetraselmis sp.</i> (Rey 3)	0.5	82	11	7	82
<i>Tetraselmis sp.</i> (Rey 2N)	0.2	43	5	95	—
<i>T. suecica</i> (TT-4 Ply 305)	0.2	27	7	41	52
<i>Tetraselmis sp.</i> (TT-9 RAL)	1.0	111	11	45	44
<i>Tetraselmis sp.</i> (TT-Caic)	7.9	1201	90	7	1
<i>T. suecica</i> (TT-3 Ply 429)	29.2	2173	90	6	2
<i>Tetraselmis sp.</i> (TT-TAH)	26.1	1458	95	2	1

*As % of total sterol; CHOL = cholesterol, 24MEC = 24-methylencholesterol; 24MC = 24-methylcholesterol.

†From Lin *et al.* (1982), as *Platymonas suecica*.‡Ballantine *et al.* (1979).

§Volkman (1986).

NA: Data not available.

24-methylcholesterol each composed over 40% of the total sterol (Table 2). Cholesterol was present in several of these isolates in small to trace quantities.

HPLC was used to determine the stereochemistry of 24-methylcholesterol at carbon 24 (Chitwood and Patterson, 1991). In each case, the 24-methylcholesterol was determined to be 24 α -methylcholesterol (campesterol). The occurrence of 24 α sterols in algae is rare. Both brassicasterol (24 β methyl) and epibrassicasterol (24 α methyl) occur in algae (Patterson, 1992) and stigmasterol (24 α ethyl) occurs in some pennate diatoms (Orcutt and Patterson, 1975; Gladu *et al.*, 1991). Of the algae reported to contain 24-methylcholesterol (Patterson, 1992), only in *Nitzschia brevivostis* was the 24-methyl group α -orientated (Gladu *et al.*, 1991). It is not possible, however, to conclude that 24 α sterols predominate in the Prasinophyceae because this is the first study of C-24 stereochemistry in the Prasinophyceae. Total sterol contents in these *Tetraselmis* strains ranged from 0.18–3.91 mg/g dry weight (Table 2).

The remaining three *Tetraselmis* isolates had a fundamentally different sterol composition, with cholesterol being the principal sterol; 24-methylenec-cholesterol and 24-methylcholesterol also were present in these isolates in amounts ranging from 1 to 7% percent of total sterol. The total sterol content of these three isolates (1201–2173 fg/cell or 7.85–29.2 mg/g dry weight) was much greater than that of the isolates with C28 sterols predominant (Table 2). The small amounts of 24-methylcholesterol from the three cholesterol-dominated isolates did not permit determination of C-24 stereochemistry in those isolates. This is the first report of cholesterol as the principal sterol in a prasinophyte.

The principal sterols of most other Prasinophyceae are 24-methylenec-cholesterol and 24-methylcholesterol, although some species of *Pyramimonas* contain remarkably different sterols (Volkman, 1986; Patterson *et al.*, 1992). Possible taxonomic utility is suggested by the apparent separation of the 11 isolates in Table 2 into groups with different predominant sterols: (1) 24-MEC, (2) 24-MC, (3) an approximately even mixture of 24-MEC and 24-MC, (4) cholesterol. The taxonomic status of the genus *Tetraselmis* is currently in some question, with Lewin (personal communication) suggesting a reduction in the number of species described historically (see also Hori *et al.*, 1983, 1986). A revised taxonomic scheme will be strengthened if sterol compositions are consistent with morphological characteristics currently being evaluated. If sterol composition is taxonomically consistent, it becomes difficult to justify including the cholesterol-rich strain TT-3 Ply 429 in the same species, *T. suecica*, as the culture analyzed by Lin *et al.* (1982) and as the other Plymouth strain analyzed in the present study, TT-4 Ply 305.

From the standpoint of molluscan aquaculture, the rather abundant quantities of cholesterol in the *Tetraselmis* isolates, TT-Caic, T-3 Ply, 409, and TT-

TAH, make these isolates attractive subjects for further analysis of nutritional biochemicals and feeding studies with cultured marine animals, especially mollusks.

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